Mammalian Biology 80 (2015) 178–181

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

Short Communication

Hair samples as monitoring units for assessing metal exposure of bats: a new tool for risk assessment

Lucie Flache[a], Nina I. Becker[a], Uwe Kierdorf[b], Sezin Czarnecki[c], Rolf-Alexander Düring[c], Jorge A. Encarnação[a,*]

[a] Mammalian Ecology Group, Department of Animal Ecology and Systematics, Justus Liebig University of Giessen, Heinrich-Buff-Ring 26 (IFZ), D-35392 Giessen, Germany
[b] Department of Biology, University of Hildesheim, Universitätsplatz 1, D-31141 Hildesheim, Germany
[c] Institute of Soil Science and Soil Conservation, Justus Liebig University of Giessen, Heinrich-Buff-Ring 26 (IFZ), D-35392 Giessen, Germany

ARTICLE INFO

Article history:
Received 26 September 2014
Accepted 23 January 2015
Handled by Danilo Russo
Available online 31 January 2015

Keywords:
Chiroptera
Non-invasive monitoring
ICP-OES
Lead (Pb)
Foraging habitat

ABSTRACT

Metals occur naturally and are ubiquitous in the Earth's crust. In addition to their release from natural sources, larger amounts of metals enter the environment through use in diverse industries, and accumulate mostly in the pedosphere close to emission sources. Bat species foraging for prey insects in different cultural landscape types (urbanized areas, forests, agricultural areas, water bodies) may show site-specific trace metal concentrations, partly due to variations in exposure among habitats. As all European bat species are protected, a non-invasive method is required to monitor their exposure to potentially toxic metals in their foraging habitats. The analysis of hair samples offers such a possibility. In the present study, hair samples of *Myotis bechsteinii*, *Myotis daubentonii*, *Myotis myotis* and *Pipistrellus pipistrellus* were analyzed for cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) using ICP-OES. We found the same order of trace metals concentrations (Zn > Mn > Cu > Pb > Cd) in *M. bechsteinii* and *M. myotis*, while *M. daubentonii* (Zn > Cu > Mn > Pb > Cd) and *P. pipistrellus* (Zn > Cu > Pb > Mn > Cd) differed from the above species and from each other. The observed differences between the bat species may reflect varying metal levels in their foraging areas as well as differences in preferred prey species and metal kinetics. Analysis of trace metal concentrations in hairs as a non-invasive and cost-effective method should be included in risk assessments regarding metal toxicity in bats.

Wildlife exposure assessments are traditionally done by analyzing internal organs, for which the animals have to be sacrificed (Talmage and Walton 1991). However, in the case of threatened and protected species, an alternative approach is needed. A possible non-invasive method for evaluating the exposure to metals in terrestrial mammals is the use of hair samples.

Hair roots absorb metals from the blood stream, which are then incorporated into the growing hair (Vermeulen et al. 2009). As a consequence, levels in hair may reflect metal concentrations of internal tissues, which make hairs suitable structures for assessing metal exposure (Burger et al. 1994).

All European bat species are protected by the Berne Convention and listed in the Annexes of the European Council Directive 92/43/EEC. To maintain bat diversity, data on potential threats have to be collected. This includes monitoring the exposure of bats to toxic metals like lead. However, also metals with a known biological function, like copper, can exert toxic effects if taken up in excess amounts (Beyer and Meador 2011). Therefore, also data on the exposure to these essential trace metals are of relevance for bat conservation.

As yet, only few studies reported metal concentrations in hairs of bats (Hartmann 2000; Hickey et al. 2001; Zukal et al., 2015). Risk assessments for bat species has been based on metal concentrations in the environment (e.g. soil), the diet of bats, biota accumulation factors, daily consumed amounts, foraging distances, and no observed effect levels (NOELs) for other small mammals, such as rats or mink (Hernout et al. 2013). Certainly, direct measurements of metal levels in bat hair would be a useful addition to such risk assessments.

In the present study we determined the concentrations of cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) in hair samples of four bat species that forage for different prey in various cultural landscape types.

The study was conducted in a typical cultural landscape in central Germany (Middle Hesse) with patchily distributed urbanized, agricultural, and forested areas intersected by river valleys (Fig. 1).

* Corresponding author. Tel.: +49 0641 99 35701.
E-mail address: J.Encarnacao@bio.uni-giessen.de (J.A. Encarnação).

http://dx.doi.org/10.1016/j.mambio.2015.01.007
1616-5047/

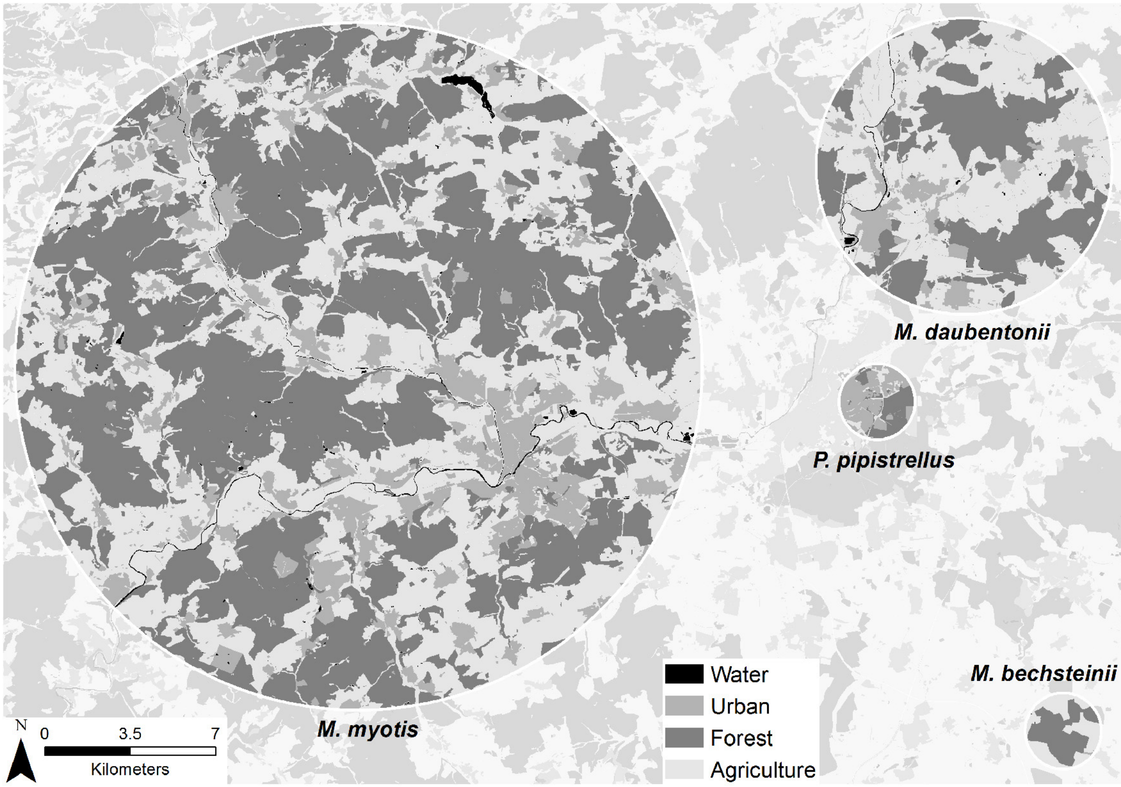

Fig. 1. Study area in Middle Hesse, Germany. Shown are the calculated surrounding areas (circles) around the roost location (circle centers) based on the maximum distance (data from literature) from the roost to the foraging area (radius) for the studied bat species *M. bechsteinii*, *M. daubentonii*, *M. myotis* and *P. pipistrellus*.

Table 1
Relevant ecological characteristics such as prey spectrum, preferred foraging habitat and maximal distance between roost and foraging habitat (distance) for the studied species (*M. bechsteinii*, *M. daubentonii*, *M. myotis* and *P. pipistrellus*).

	M. bechsteinii	*M. daubentonii*	*M. myotis*	*P. pipistrellus*
Body mass [g][1]	9–10	7–9	28–35	4–7
Body length [mm][1]	44–61	50–60	65–74	38–47
Forearm length [mm][1]	39–44	34–39	60–63	31–33
Day roosts (predominantly)[1]	Tree holes	Tree holes	Attics	Clefts in buildings
Foraging habitats	Forests[2]	Water bodies (river, ponds)[3]	Forests[4], Agricultural areas[4]	Hedges[5], Alleys[5], Lit areas[6]
Prey spectrum[7]	Diptera, Lepidoptera	Diptera (Chironomidae), Trichoptera	Coleoptera (Carabidae)	Diptera, Lepidoptera
Distance [km]	1.5[1]	6[1]	14[8]	1.5[1]
Red List status Europe[9]/Germany[10]	VU/CR	LC/LC	LC/NT	LC/LC
Annexes of the Habitats Directive[9]	II/IV	IV	II/IV	IV

VU = vulnerable, CR = critically endangered, LC = least concern, and NT = near threatened. References: [1]own data; [2]Becker and Encarnação 2012; [3]Encarnação et al. 2004; [4]Arlettaz 1999; [5]Verboom and Huitema 1997; [6]Rydell 1989; [7]Vaughan 1997; [8]Güttinger 1997; [9]Temple and Terry 2007; [10]Rote Liste Deutschland 2009.

Within this region, the four studied bat species *Myotis bechsteinii*, *Myotis daubentonii*, *Myotis myotis* and *Pipistrellus pipistrellus* occur in stable populations. Relevant biological and ecological characteristics of the study species are summarized in Table 1.

Bats (*M. bechsteinii*, $n = 14$; *M. daubentonii*, $n = 9$; *M. myotis*, $n = 12$; *P. pipistrellus*, $n = 8$) were captured between April and July of 2011 in their foraging habitats using mist nets or at their roost sites using a funnel-shaped basket trap. Juveniles were distinguished from adults based on the presence of unfused and translucent phalangeal epiphyses (Anthony 1988), and only adult individuals were used for this study. Dorsal hair (1–5 mg per animal) was clipped from the fur, immediately transferred into sterile disposable tubes and stored at room temperature.

Capture, handling, and hair sampling of the bats were performed with the permission of the nature conservation authority and the animal care authority of the administrative district of Giessen, federal state of Hesse, Germany.

Bat hair samples were cleaned prior to analysis to remove external metal contaminations using a method described by Hickey et al. (2001). Hair samples were then dried to constant mass and 500 μL of 65% (w/v) nitric acid was added to each sample. Samples were extracted for 5 min at 200 W followed by another 5 min at 300 W. Extracts were made up to volume with deionized water and stored at 4 °C until analysis. Blanks were treated in the same way as the samples.

Trace metal concentrations in hair samples were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Agilent 720ES). Miniaturized microwave closed vessel extraction (μMAE) was adapted from Czarnecki and Düring (2014) and performed with a StarT-1500 microwave oven (MLS, Leutkirch, Germany). The reagents used for determination of trace metals were all of analytical-reagent grade certified for impurities.

By appropriate dilutions of ICP multielement standard, element solutions were prepared to determine the accuracy of the ICP-OES measurement. For calibration of ICP-OES measurements an external 6-point calibration curve (range: 0.10–5.00 μg/g) was used for each metal. Certified reference material (CRM) "397" ("human hair", National Institute for Environmental Studies, Japan) was used